Therapeutic potential of flavonoids in spinal cord injury

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Abstract

Spinal cord injury (SCI) is a catastrophic event that can profoundly affect a patient's life, with far-reaching social and economic effects. A consequential sequence of SCI is the significant neurological or psychological deficit, which obviously contributes to the overall burden of this condition. To date, there is no effective treatment for SCI. Therefore, developing novel therapeutic strategies for SCI is highly prioritized. Flavonoids, one of the most numerous and ubiquitous groups of plant metabolites, are the active ingredients of traditional Chinese medicine (TCM) such as Scutellaria baicalensis Georgi (Huang Qin) or Ginkgo biloba(Ying Xin).Accumulated research data show that flavonoids possess a range of key pharmacological properties such as anti-inflammatory, antioxidant, anti-tumor, anti-viral, anti-cardiovascular disease, immunomodulatory, and neuroprotective effects. Based on this, the flavonoids show therapeutic potential for SCI diseases. In the following article, we will review the pharmacological properties of different types of flavonoids for treatment of spinal cord injury diseases, and potential underlying biochemical mechanisms of action will also be described.

Key words: Flavonoids; Spinal cord injury; Pharmacology; Anti-inflammatory; Antioxidants; Anti-apoptosis; Neuroprotection.

Introduction

In modern society, the estimated annual global incidence of spinal cord injury (SCI) is approximately 15–40 cases per million and is increasing (Singh et al., 2014; Sekhon and Fehlings, 2001). SCI is a catastrophic event that can profoundly affect a patient's life, with far-reaching social and economic effects(Sekhon and Fehlings, 2001). A consequence of SCI is the significant neurological or psychological deficit, which obviously contributes to the overall burden of this condition (Evaniew et al., 2015). Along with the development of neurobiology, materials science, pharmacology, and other related sciences, great progress has been made in the prevention and treatment of SCI.

Significant progress has been made to relieve the symptoms of SCI and the suffering of patients, which are achieved by preventing injury progression (Silva et al., 2014; Zhang et al., 2016). At present, in the early phase, the conventional treatment of spinal cord injury in clinic is surgical therapy combined with high doses of methylprednisolone (MP). The surgical procedures are able to decompress the spinal cord and restore the normal anatomy of the spine and stability(Ahuja, Martin, and Fehlings, 2016).Although MP has many advantages such as maintaining the blood-spinal cord barrier, enhancing spinal cord blood flow, and limiting the inflammatory response(Suberviola et al., 2008), potential harms of MP include risks of infections and gastrointestinal bleeding, potentially leading to increased mortality. Therefore, most current guidelines do not recommend routine administration of MP for acute SCI(Hurlbert et al., 2015; Hurlbert, 2014; Walters et al., 2013). Meanwhile, various other novel strategies for SCI repair have emerged and received considerable research attention, including drug therapy such as Gangliosides(Chinnock and Roberts, 2005), Erythropoietin (EPO)(Nekoui et al., 2015), Minocycline(Aras et al., 2015), Vitamin B12(Petchkrua et al., 2003), Tacrolimus(Pan et al., 2013); cell therapy such as transplantation of neural stem cells(Cheng et al., 2016), bone marrow-derived mesenchymal stem cells(Yousefifard et al., 2016), adipose-derived mesenchymal stem cells(Kim et al., 2015), Schwann cells(Oraee-Yazdani et al., 2016), olfactory ensheathing cells(Li et al., 2015); gene modification technique therapy such as nerve growth factor (NGF)(Zhai et al., 2015), neurotrophin-3(Hanna et al., 2016), brain-derived neurotrophic factor (BDNF)(Wang, Liao, and Li, 2010), glial cell line-derived neurotrophic factor (GDNF)(Chou et al., 2014),or ciliary neurotrophic factor(Abbaszadeh et al., 2015).However, it is worth noting that most of the treatments are still at the research stage, and are not ready for use in the clinic. Thus, the treatment of SCI is currently a significant challenge in the clinic and in research around the world. To date, there is no majorly effective treatment for SCI(Zhao et al., 2011).

As a productive natural resource for drug discovery, Traditional Chinese Medicine (TCM),has many thousands of years of history in clinical applications in China and other Asian countries, plays a role in complementary and alternative medical systems, and has an advantage for treatment of SCI due to a large number of experimental studies(Liu et al., 2015; Boots, Haenen, and Bast, 2008; Manach et al., 2005). Although TCM therapy could not replace surgical procedures, it could be play a supportive role in treating SCI. Scutellaria baicalensis Georgi (Chinese name Huang Qin), Quercetin(Chinese name Hu Pi), Puerarin (Chinese name Ge Gen), Safflower Yellow (Chinese name Hong Hua Huang), -Epigallocatechin gallate (Chinese name Er Cha Su), Anthocyanins (Chinese name Hua Qing Su), Ginkgo biloba (Chinese name Ying Xin), and

numerous others. TCM has provided classic herbal medicine for treating spinal cord injury diseases in the clinic for many years. It was found that the main active components are flavonoids by using modern pharmacological discovery. Flavonoids,one of the most numerous and ubiquitous groups of plant metabolites, have anti-inflammatory, antioxidant, neuroprotective physiological activity as demonstrated in experimental studies. In addition, a number of studies have found that flavonoids have therapeutic potential in SCI. However, the molecular mechanisms involved the effects shown by TCM still remain unclear, and it can be challenging to systematically identify them using modern pharmacological and biochemical techniques(Liu et al., 2015).In the following paragraphs, we will review the pharmacological actions of different types of flavonoids for the treatment of spinal cord injury diseases, and some potential underlying biochemical mechanisms of action will also be described.

Pathophysiology of SCI

Patients with spinal cord injury often experience severe loss of function and a profoundly impaired quality of life. More and more research has focused on elucidating the mechanisms and complex pathophysiologic processes of SCI. Pathophysiological events occurring after SCI include a primary mechanical injury and a secondary injury.

A primary mechanical injury often results from a mechanical impact to the spine, concomitant with or followed by compression, contusion, stretching or kinking of the spinal cord(Stahel, VanderHeiden, and Finn, 2012).This phase refers to the immediate post-injury period, and this process is inevitable. The damage level of this stage is very difficult to control, which depends on the intensity of force and the compression time on the spinal cord(DeVivo, Go, and Jackson, 2002). Therefore, we have no treatment for a primary mechanical injury.

A secondary injury mediated by multiple processes after the initial trauma(Amar and Levy, 1999). The time course of this process ranges from minutes to weeks following SCI and leads to further damage. The outcomes of SCI depend mainly on the extent of secondary damage produced by a series of cellular and molecular events such as inflammation(Popovich, Wei, and Stokes, 1997), free radical production(Hall, 1993, 1993), vascular events(Mautes et al., 2000), glutamate excitotoxicity(Dong and Wang, 1998), and apoptosis(Liu et al., 1997), which inevitably induce neuronal and glial cell death at and beyond the site of injury(Zhao et al., 2011).

The secondary stage can cause a series of events, for example, an inflammatory response induces higher extravasation of leukocytes and further tissue damage of the surrounding original injury site(Yang et al., 2005; Donnelly and Popovich, 2008).Moreover, several studies have demonstrated that inflammation also plays an important role in blocking neural tissue repair; free radical formation and lipid peroxidation cause oxidative death of the spinal cord neurons and reduce spinal cord blood flow that leads to edema and a continuation of the inflammatory response(Toborek et al., 1999); vascular changes lead to edema, necrosis, and ischemia(Bareyre and Schwab, 2003); glutamate excitotoxicity leads to further neuronal cell death; apoptosis can cause programmed cell death of neurons, oligodendrocytes, microglia, and, perhaps, astrocytes after SCI(Beattie, Farooqui, and Bresnahan, 2000). Moreover, this process will last for a long time(Wilson and Fehlings, 2014). Without immediate treatment, SCI will lead to neurological impairments in motor function, affect the function of brain regions, as well as the development of pain syndromes and mood disorders such as depression(Yiu and He, 2006). Therefore,

developing novel therapeutic strategies for preventing this series of events of the secondary stage of SCI is highly prioritized.

Flavonoids

Flavonoids are in the most common and widely distributed group of plant phenolic compounds, occurring virtually in all plant parts, particularly the photosynthesizing plant cells. Meanwhile, flavonoids in food are generally responsible for colour, taste, prevention of fat oxidation, and protection of vitamins and enzymes.

Flavonoids are extracted from plants that have been used for the treatment of neurovascular and spinal diseases for centuries in Chinese medicine, and more experience is continuously gained about their significant neuroprotective properties(Sang et al., 2004; Lee et al., 2012; Wang et al., 1997; Zhao et al., 2006). Flavonoids are hydroxylated phenolic substances and are known to be synthesized by plants in response to microbial infection. A number of studies show flavonoids compounds have anti-inflammatory(Thao et al., 2016), antioxidant(Baharfar, Azimi, and Mohseni, 2015), anti-tumor(Srivastava et al., 2016; Saeed et al., 2015), anti-viral(Yin et al., 2014), anti-cardiovascular disease(Macready et al., 2014), and immunomodulatory effects(Sternberg et al., 2009).

Flavonoidscontaining a 2-phenyl chromone structure are common in nature(Tsuji et al., 2013). They make up a large group of polyphenolic compounds that have a benzo-γ-pyrone structure and are ubiquitously present in plants. According to the oxidation degree of the structure of the three carbon bonds (C3) and the connection position of the B ring, the flavonoid compounds can be classified into the following categories *(Fig 1)*:flavones, flavonol, isoflavone, chalcone, aurones, flavanes, anthocyanidins, biflavone and other two-hydrogen derivatives(Gonzales et al., 2015; Sternberg et al., 2009). Flavonoids occur as aglycones, glycosides, and methylated derivatives. The basic flavonoid structure is aglycone. The position of the benzenoid substituent divides the flavonoid class into flavonoids (2-position) and isoflavonoids (3-position). Flavonols differ from flavanones by a hydroxyl group at the3-position and aC2–C3 double bond(K.R.Narayana, 2001). Flavonoids are often hydroxylated in positions 3, 5, 7, and 2. When glycosides are formed, the glycosidic linkage is normally located in positions 3 or 7 and the carbohydrate can be L-rhamnose, D-glucose, glucorhamnose, galactose, or arabinose(Middleton, 1984).

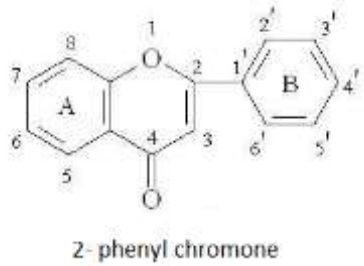


figure 1 The basic structure of flavonoids

Flavonoids have extensive biological properties thatpromote human health and help reduce the risk of diseases. The isoflavanglabridin, a major polyphenolic compound found in Glycyrrhiza glabra, inhibits low-density lipoprotein (LDL) oxidation via a mechanism involving scavenging of

free radicals(Fuhrman et al., 1997). Flavonoids from Green or black tea may lower blood cholesterol concentrations and blood pressure, thereby providing some protection against cardiovascular disease. Flavonoids are also known to influence the quality and stability of foods by acting as antioxidants(Craig, 1999; Kumar et al., 2012). Flavonoids contained in berries may have a positive effect against Parkinson's disease and may help to improve memory in elderly people. Antihypertensive effect has been observed in hypertensive rats(Li et al., 2005).

Pharmacology of flavonoids in SCI

Flavones and Dihydroflavones

The key source of flavones and dihydroflavones *(Fig2)* is the Scutellaria baicalensis Georgi (Huang Qin),an important medicinal herb in China. It has been widely used for the treatment of inflammatory diseases and ischemic stroke for thousands of years. Baicalin (7-glucuronic acid, 5, 6-dihydroxyflavone,BC)(*Fig2*), a flavonoid compound isolated from Huang Qin, has been proven to possess anti-oxidant, anti-inflammatory, and anti-apoptotic properties. Importantly, BC can pass through the blood–brain barrier (BBB) into the central nervous system (CNS) and could be a novel and promising therapeutic agent for human spinal cord injury(Shang et al., 2006; Zhang et al., 2006; Gao et al., 1999).

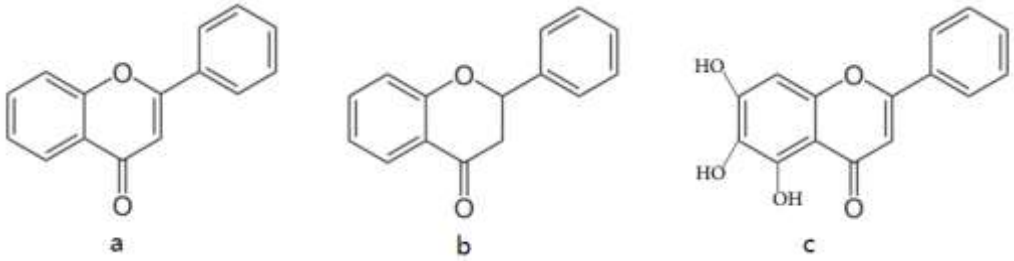


Fig 2 Structure of Flavones and Dihydroflavones: a. generic molecular structure of Flavones; b. generic molecular structure of Dihydroflavones; c. structure of Baicalin.

Anti-inflammatory properties

The inflammatory reaction within the spinal cord and generation of proinflammatory cytokines at the site of injury, such as tumor necrosis factor-α (TNF-α) and nuclear factor-κB (NF-κB), has been shown to be involved in regulating the precise cellular events after SCI in a SCI rats model(Shang et al., 2006; Smith et al., 2010; Lin et al., 2010; Zhang et al., 2009). The generation of inflammatory cytokines is regulated by the transcription factor NF-κB in rat models with different cellular inflammatory reactions(Streit et al., 1998). It was found that by treatment with BC, the expression of NF-κB mRNA and TNF-α mRNA significantly decreased, and the extent of spinal cord damage was also alleviated to some extent at the same time in a compression spinal cord injury rat model(Cao et al., 2010). Other studies found that intrathecalbaicalin significantly inhibited histone deacetylase 1 (HDAC1) phosphorylation in nerve ligation of rat spinal cords; which suggests that BC might directly affect the HDAC1 protein structure by modification at the posttranslational level to inhibit neuroinflammation in the spinal cord of rats with spinal nerve ligation(Cherng et al., 2014). Therefore, BC has helpful effects in treating the injured spinal cord.

Antioxidant properties

It has been shown that production of free radicals and reactive oxygen species induced by SCI contribute to the disruption of endothelium and the opening of the blood–spinal cord barrier (BSCB), whereas anti-oxidant compounds attenuate edema formation and cell injury after SCI in rats(Sharma et al., 2006). BC is a scavenger of free radicals in cultured human neuroblastoma SH-SY5Y cells (Gao et al., 1999). The BC treatment significantly reduces the increased malonaldehyde(MDA) values and increases the glutathione (GSH) levels after SCI in an animal model, suggesting that the protective effect of BC may be related to the inhibition of free radical and oxidant formation.

Anti-apoptotic properties

Recent studies have demonstrated that BC possesses potent anti-apoptotic properties and attenuates cerebral ischemia injury in rats(Shang et al., 2006; and et al., 2010). Key anti-apoptotic signaling molecules in neurons are Bcl-2, Bax and the Bcl-associated death promoter in transgenic mice(Martinou et al., 1994). BC reduces the expression of Bax and increases the expression of Bcl-2 compared with SCI saline-treated rats. In addition, caspase-3 is activated during the early phase of apoptosis and activation of caspase-3 is part of the apoptosis process by enhancing the cleavage of DNA, nuclear lamins, and cytoskeletal components. Taken together, these properties suggest that BC prevents the loss of anti-apoptotic signaling and reduces the pro-apoptotic pathway activation.

Neuroprotective properties

SCI results in a significant increase in lipid peroxidase, nitric oxide, TNF-α, interleukin-1β (IL-1β), and Bax levels, whereas expression of bcl-2 and caspase-3 are significantly reduced by treatment with chrysin. This shows that chrysin is a flavones containing antioxidant, and its anti-apoptotic property promotes the subsequent recovery of both motor and sensory functions via modulation of endogenous biomarkers and neuronal apoptosis to reduce the neurological deficits caused by SCI in a rat model of SCI(Kandhare et al., 2014).

Flavonol and Dihydroflavonol

Representative members of the flavonol and dihydroflavonol groups are rutin, quercetin, and Silymarin *(Fig3)*. Rutin, a flavonoid obtained from plants, has been shown to be effective in conditions involving inflammation and oxidative stress. Studies show flavonoid compounds have anti-inflammatory, antioxidant, anti-tumor, anti-viral, anti-cardiovascular disease, and immunomodulatory effects(Matsunaga et al., 2000; Aruna et al., 2014; Song et al., 2015). Studies indicate that rutin improves spatial memory and neurological function in an Alzheimer's disease transgenic mouse model, and reduces neurodegeneration in the periphery of cortical injury(Xu et al., 2014; Rodrigues et al., 2013). A compound with anti-oxidative and anti-inflammatory properties such as quercetin is a potentially useful therapeutic treatment for SCI. The experimental results also suggest a protective role for quercetin in the bladder against SCI-induced tissue damage by its ability to downregulate pro-inflammatory cytokines and to inhibit neutrophil infiltration and apoptosis, and to balance the oxidant-antioxidant status(Schultke et al., 2010). Silymarin, a European herbal drug, has been used for a long time by patients suffering from liver diseases of different etiology. Silymarin, purified from milk thistle

silybummarianun, consists of a mixture of 7 flavonolignans and polyphenols. Silymarin has been identified in several in vitro and in vivo models as antioxidants and radical scavengers(Feher and Lengyel, 2012; Ramasamy and Agarwal, 2008).

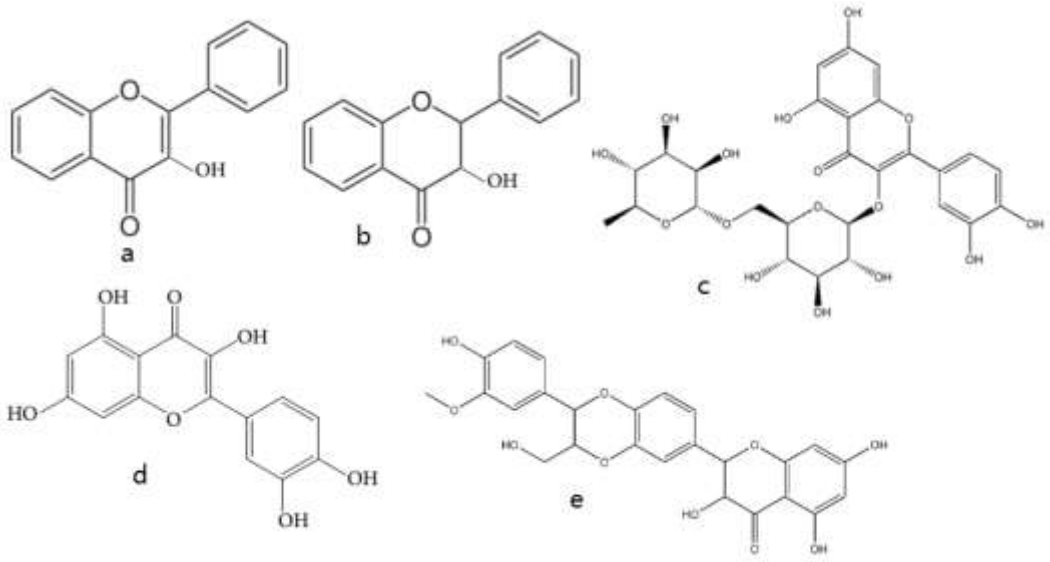


Fig3 Structure of Flavonol and Dihydroflavonol: a. generic molecular structure of Flavonol; b. molecular structure of Dihydroflavonol; c. structure of rutin; d. structure of quercetin; e. structure of Silymarin.

Anti-inflammatory properties

Studies show that rutin suppresses NLRP3 (NACHT, LRR, and PYD domains-containing protein 3) inflammasome activation and decreases levels of IL-1β, IL-18, TNF-α and reduces ROS and MDA productions by histopathology at the 72 h after SCI in a rat model(Zendedel et al., 2016). Quercetin has been shown to reduce plasma levels of pro-inflammatory cytokines (TNF-α, IL-6, IL-1β), myeloperoxidase (MPO) levels, and ameliorates nano zinc oxide particle-induced nephrotoxicity in rats after SCI in an animal model(Impellizzeri et al., 2015; Schultke, Griebel, and Juurlink, 2010).

Anti-oxidative properties

Studies showed that rutin control oxidative stress by inhibition of NLRP3 oxidant activation in a rat model of SCI(Wu et al., 2016; Schroder and Tschopp, 2010). This indicates that the activation of the Protein 38 mitogen-activated protein kinase/inducible nitric oxide synthase (p38MAPK/iNOS)signaling pathway is involved in the secondary injury mechanism post-SCI(Lawrence et al., 2008),quercetin can regulate secondary oxidative stress following SCI by directly clearing oxidants and inhibiting activation of thep38MAPK/iNOS pathway in SCI rats(Wu et al., 2016). In another study, investigators treated mixed neuronal/glial cell cultures with Silymarin. The results show that Silymarin inhibits cell proliferation and protects mixed glial cell cultures against peroxide toxicity. Protein kinase C (PKC) or NF-kB inhibitors blocked Silymarin protection against H_2O_2 toxicity. Silymarin protected mixed cortical and spinal neuronal/glial cells against peroxide challenge, spinal cord neuronal/glial cell cultures from lipopolysaccharides (LPS)induced inflammation, and glial cell cultures and microglia from LPS stimulation or peroxide toxicity in primary neuronal/glial cell cultures and *in vivo*(Tsai et al., 2010).

Anti-apoptotic properties

In addition, rutin can prevent SCI-induced programmed cell death in SCI rats model (Zhang and Ma, 2015). A study revealed that rutin reduced programmed cell death by influencing the down-regulation of the NLRP3 in an alcohol and cerulein-induced rat model of pancreatitis(Aruna, Geetha, and Suguna, 2014). Jeong et al. reported that rutin inhibited myocardial ischemia/reperfusion-induced apoptosis via extracellular regulated protein kinases 1/2 (ERK1/2) and phosphatidylinositol 3 kinase/ protein kinase B (PI3K/Akt) signaling in vitro(Jeong et al., 2009). Caspase 3 has been associated with apoptosis in spinal cord injury, and quercetin treatment counteracted apoptotic cell death as measured by immunoblotting of the cleaved caspase 3 and caspase 3 activity. Moreover, quercetin inhibited the phosphorylation of the c-Jun N-terminal kinase/stress-activated protein kinase in rats(Wei et al., 2012; Dasari et al., 2007) (JNK/SAPK)and p38MAPK that are involved in the inhibition of cell growth as well as the induction of apoptosis in an animal model(Angeloni and Hrelia, 2012; Cevik et al., 2013).

Neuroprotective properties

Rutin exhibits neuroprotective effects through antioxidative and anti-inflammatory activity. In vitro, rutin reduces nitric oxide and pro-inflammatory cytokine production in SH-SY5Y neuroblastoma cells(Wang, Wang, et al., 2012). Furthermore, rutin attenuated 6-hydroxydopamine-induced neurotoxicity via improving antioxidant enzyme levels and reducing lipid peroxidation in PC-12 cells(Magalingam, Radhakrishnan, and Haleagrahara, 2013). Moreover, in vivo, rutin has been demonstrated to enhance the neurotrophic effect by reduction of macrophage inflammatory protein-2 (MIP-2) and p-Akt expression and matrix metallopeptidase-9activation in SCI rats(Zhang and Ma, 2015). Furthermore, rutin augments Basso Beattie Bresnahan scores and decreased spinal cord water content of SCI rats. Following quercetin treatment of 0.2 mg/kg per day, the mean values of motor activity scores and critical angle of inclined plane test were significantly increased that even brief exposure to quercetin had some neuroprotective effect in an animal model of spinal cord compression injury(Schultke et al., 2010).

Isoflavone and Dihydroisoflavone

A representative member of the Isoflavone and Dihydroisoflavone group is Puerarin (*Fig4*). Puerarinis extracted from Radix puerariae that has been used for the treatment of neurovascular and spinal diseases for centuries in Chinese medicine, and its significant neuroprotective properties have attracted attention, particularly in the treatment of spinal ischemic damage(Sang et al., 2004; Xing et al., 2011). Available reports on the neuroprotective mechanisms of puerarin involve superoxide dismutase activity(Nwose and Ewing, 2009), lipidperoxidation, fibrinolysis, inflammatory responses, and cell apoptosis(Xu et al., 2005).

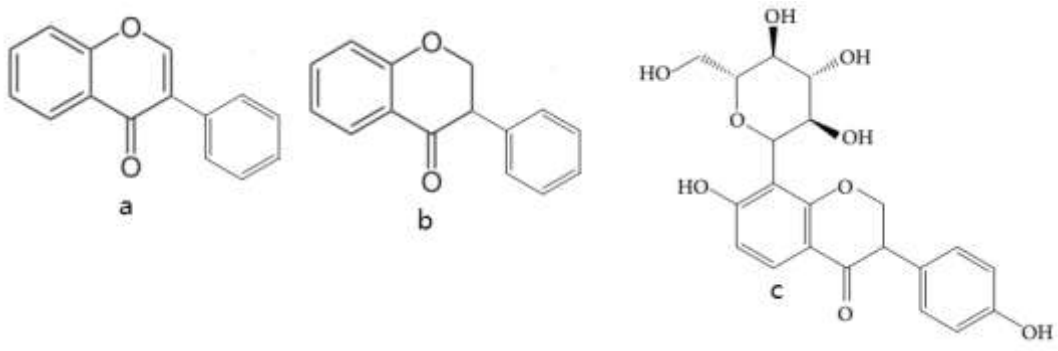


Fig 4 Structure of Isoflavone and Dihydroisoflavone: a. the generic molecular structure of Isoflavone; b. the generic molecular structure of Dihydroisoflavone; c. structure of Puerarin.

Neuroprotective properties

Previous studies have shown a neuroprotective effect of puerarin on spinal ischemic damage in an animal model. Furthermore, puerarin can also increase spinal blood flow in Clinical observation(Zhou et al., 2006), and reduce spinal cord injury in an ischemia–reperfusion rabbit model. A recent study has demonstrated that puerarin reduces hypoxia inducible factor 1 and TNF-α levels, apoptosis, and neutrophil activation, resulting in a reduction of the infarct volume in ischemia–reperfusion brain injury in rats(Chang et al., 2009). The neuroprotective mechanism of puerarin against spinal ischemia–reperfusion injury may involve the upregulation of thioredoxin mRNA and reduction of apoptosis in the spinal cord ischemia-reperfusion injury rat model(Tian et al., 2011). The neurological deficit caused by SCI/ reperfusion injury (RI) was associated with glutamate release and elevation of glutamate RNA expression in acute spinal cord injury rat model(Tian, Xu, et al., 2013).

Chalcone

The representative member of the chalcone group is Safflower Yellow (SY; *Fig5*), which is extracted from the flowers of the plant safflower (Carthamus tinctorius) and in traditional Chinese medicine it has been extensively used for the treatment of cardio-cerebrovascular diseases. Hydroxysafflor yellow A (HSYA) (*Fig5*), which is the main chemical component of the safflower yellow pigments, has been demonstrated to have potent antioxidative effect in vitro(Jin, Li, and Wu, 2004). SY can promote blood circulation, remove blood stasis, and thereby improve capillary circulation at the site of tissue injury. Additionally, a previous study indicated that SY alleviates the injured tendon adhesion and inflammatory reaction and promotes the repair of injured tendons(Shan et al., 2010). Recent studies revealed that HSYA could alleviate ischemia-reperfusion injury of the lung, heart and brain via scavenging of free radicals(He et al., 2008; Wei et al., 2005).

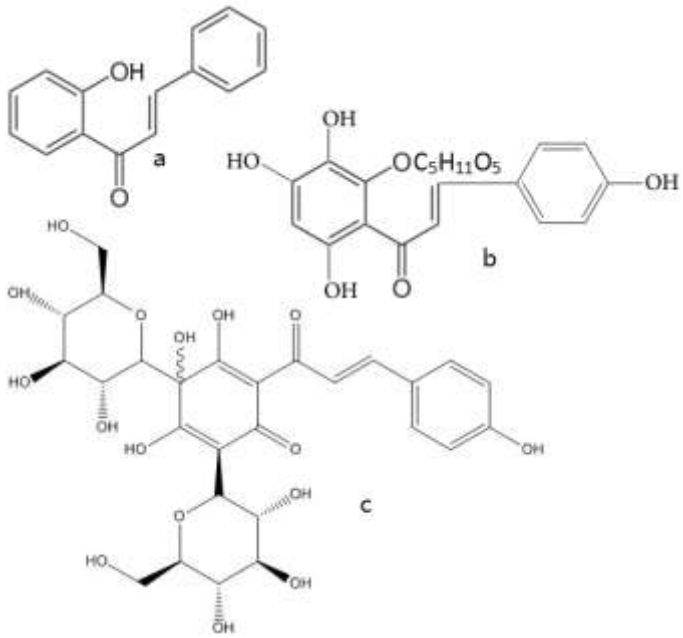


Fig 5 Structure of chalcone: a. the generic molecular structure of chalcone; b. structure of Safflower yellow (SY); c. structure of Hydroxysafflor yellow A (HSYA).

Anti-inflammatory properties

IL-8 is an important cytokine, which plays a key role in the inflammation response(Kunihara et al., 2000). One study shows that SYinhibits IL-8 expression and the inflammatory response induced by SCIRI in a rabbit spinal cord ischemia reperfusion(IR) injury animal model(Zhou et al., 2013).

Antioxidant properties

Safflower and its extracts play an important role in of inhibiting lipid peroxidation and clearing oxygen free radicals in rats(Ma et al., 2015). Safflower injection can significantly activate glutathione peroxidase and superoxide Dismutase (SOD) and decrease MDA levels in myocardium ischemia reperfusion injury. These results indicate that SY could play an important role in spinal cord protection by relieving lipid peroxidation in a rabbit spinal cord ischemia reperfusion injury animal model(Zhou et al., 2013).

Anti-apoptotic properties

A recent study confirmed that activation of caspase-3 expression correlates with levels of cell apoptosis after SCI, suggesting that caspase-3 could be used as biochemical index in assessing post-ischemic spinal cord injury in the clinic(Lin et al., 2012). The results demonstrate that inhibiting caspase-3 expression may represent a mechanism by which SY confers protection against SCI in a rabbit spinal cord ischemia reperfusion injury animal model(Zhou et al., 2013). HSYA significantly reduced the number of apoptotic cells in the anterior horn of the spinal cord. This protective effect of HSYA against neuronal apoptosis may be related to its antioxidative effect because a major mechanism of ischemia/reperfusion induced apoptosis is attributed to ROS release in a rabbit spinal cord ischemia reperfusion injury animal model (Shan et al., 2010).

Neuroprotective properties

One study provided direct in vivo evidence that HSYA confers neuroprotection through its antioxidative action. Moreover, the neuroprotective effect of HSYA might also be attributed to its inhibition of thrombosis formation and platelet aggregation, its regulation of the prostaglandin I2/thromboxane ratio and blood rheological changes, as well as its suppression of inflammatory responses in a ischemic injury rat model(Zhu et al., 2005; Ye and Gao, 2008).

Flavanes

A typical flavane is the (-)-Epigallocatechingallate (EGCG) (*Fig6*), the most abundant active component of the tea catechins and thought to be responsible for most of the biological activity of green tea extracts(Kimura et al., 2002). EGCG is known for its potent antioxidant and anti-inflammatory properties(Kim et al., 2003). EGCG also has been shown to have some protective effects against neuronal damage. A recent study shows that EGCG could attenuate neuronal apoptosis and improve locomotor function after SCI in rats(Khalatbary and Ahmadvand, 2011).

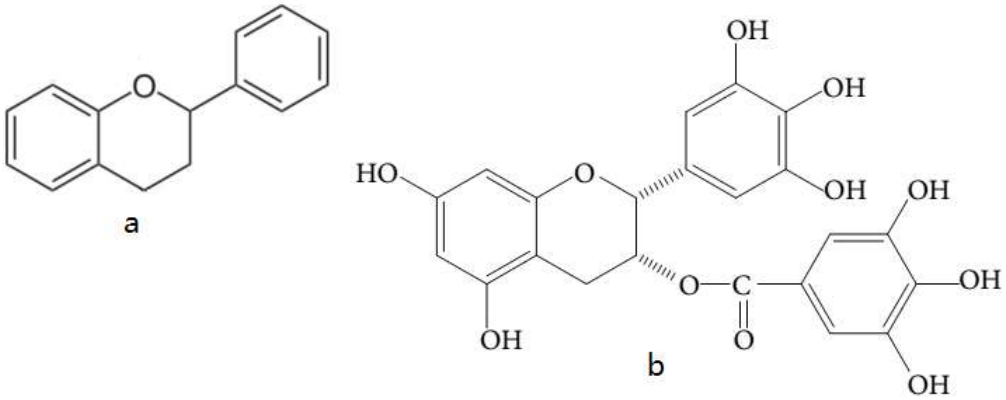


Fig 6Structure of flavane: a. the generic molecular structure of flavane; b. structure of (-)-Epigallocatechingallate (EGCG).

Anti-inflammatory properties

A recent study showed that MPO activity significantly decreased in EGCG-treatment groups. Attenuated TNF-α, IL-1β, Nitrotyrosine, iNOS, cyclooxygenase-2 (COX-2), and poly-ADP-ribose polymerase (PARP) expression was obseved in the EGCG treated rats. Also, EGCG attenuated myelin degradation. It appears that EGCG may be effective in protecting rat spinal cord neurons from secondary damage by modulating the inflammatory response in a spinal cord trauma rat model(Khalatbary and Ahmadvand, 2011).

Antioxidant properties

Another study showed that EGCG modulates the levels of $O^{2-}$and SOD. In addition, the MDA levels in the EGCG treatment groups were significantly lower than those in the trauma group after SCI in an SCI rat model(F. Deng, 2011; Khalatbary et al., 2010).

Anti-edema properties

The results of a different study imply that EGCG has an anti-edema effect after acute SCI.

The anti-edema effect could be the result of down-regulation of the expression of Aquaporin-4 (AQP4) protein levels. Also EGCG may reduce astrogliosis after SCI through down-regulation the expression of glial fibrillary acidic protein (GFAP) levels in a SCI rat model(Ge et al., 2013).

Anti-apoptotic properties

EGCG inhibits the expression of the pro-apoptotic protein Bax and increases that of the anti-apoptotic protein Bcl- 2, thereby providing a molecular mechanism for the neuroprotective activity of EGCG. The number of TUNEL-positive cells at the spinal lesion site of EGCG-treated rats was significantly lower than that in the trauma group in the contusive spinal cord injury rat model(Khalatbary et al., 2010).

Neuroprotective properties

Intravenous infusion of EGCG in acute or chronic phase following SCI in rats promotes significant locomotor recovery as measured by the standard motor tests and The Louisville Swim Scale tests. EGCG-treated SCI animals significantly improve on the tactile allodynia reflex and mechanical nociception threshold(Renno et al., 2014). Moreover, 4 weeks after SCI, the expression of neurotrophic factors BDNF and GDNF in the spinal cords of EGCG-treated animals were higher than in the control group(Tian, Han, et al., 2013). Treatment with EGCG modulates the expression of growth associated protein-43 (GAP-43) and GFAP levels and protects neurons from degeneration caused by contusion lesion in the spinal cord in rats(Renno et al., 2014).

Anthocyanidins

Anthocyanins (*Fig7*) are natural pigments belonging to the flavonoid family and are present in fruits and vegetables. They are known to have powerful antioxidant effects, anti-carcinogenic properties and potent cardioprotective effects. They can also inhibit inflammation(Romero et al., 2008; Steed and Truong, 2008; Xu and Chang, 2009). Among anthocyanins, cyanidin 3-O-β-glucoside (C3G) (*Fig7*) has the highest protective effects as a scavenger of active oxygen species in hepatic ischemia-reperfusion damage and cerebral ischemia models. Two studies were published on the pre-/post-treatment of C3G which showed significant improvement of neurological recovery and a decrease of infarction volume in cerebral ischemia rat models(Kang et al., 2006; Shin, Park, and Kim, 2006). Bog bilberry is a low-growing deciduous shrub of the Ericaceae family of plants. Berries of the Ericaceae family are known to contain organic acids, vitamins, glycosides, and anthocyanins and have been reported to have antioxidant activity. Bog bilberry anthocyanin extract (BBAE) (*Fig7*)has been shown to have antioxidant activity(Kim et al., 2009).

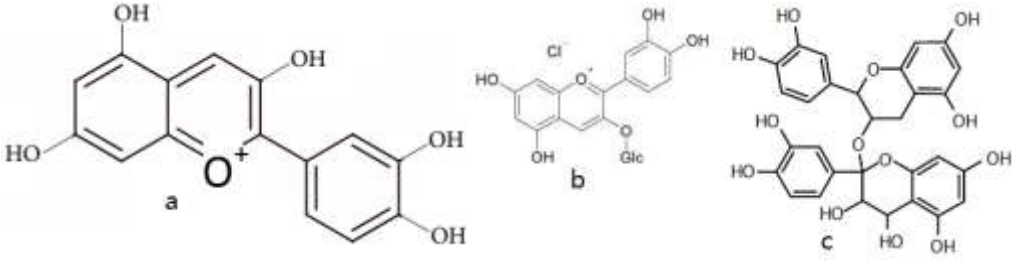


Fig 7 Structure of Anthocyanins: a. generic molecular structure of Anthocyanins; b. structure

of cyanidin 3-O-β-glucoside (C3G); c. structure of Bog bilberry anthocyanin extract (BBAE).

Anti-inflammatory properties

BBAE treatment reduced the expression the key cytokines TNF-a, IL-1β, and IL-6 in a rat model of SCI(Wang, Ma, et al., 2012).

Antioxidant properties

The main therapeutic role of C3G is the scavenger of ROS. C3G significantly reduced the production of superoxides in lesion peripheries of SCI in the traumatic SCI rat model(Kim et al., 2011).

Neuroprotective properties

In addition, C3G decreased the lesion volume and protected motor neurons in the anterior horn of lesion peripheries 14 days after SCI in rats(Kim et al., 2011).These observations show that the BBAE therapy could exert protective effect on the neurons and promotes neuronal survival after SCI. In addition, BBAE can reduce myelin axonal loss, glial scars formation, and astrocyte proliferation after SCI in a rat model(Wang, Ma, et al., 2012).

Biflavone

A representative member of the biflavonoid group (*Fig 8*) is the Ginkgo biloba extract (EGb761),a complex mixture of ingredients with broad pharmacological effects on the CNS. Recent studies using the SCI model revealed that EGb761 is involved in the protection of spinal cord neurons in ischemic injury in vivo*(Mechirova et al., 2009)* and hydrogen peroxide-induced spinal cord neuronal death *in vitro*. The neuroprotective effects of EGb761 include scavenging free radicals, lowering oxidative stress, reducing neural damage, preventing platelet aggregation, and anti-inflammation(Wang, Ma, et al., 2012; Jiang et al., 2009; Kotakadi et al., 2008). Ginkgolides B is the active constituent that has attracted the most attention in recent years. Ginkgolide B can improve hemorrhage, edema, necrosis, and inflammatory cell infiltrates in the injured spinal cord(Zhang et al., 2016).

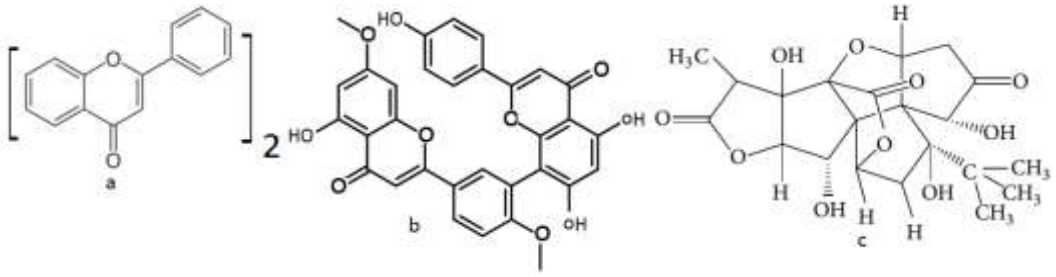


Fig 8 Structure of biflavonoid: a. generic molecular structure of biflavonoid; b. structure of Ginkgo biloba extract (EGb761); c. structure of ginkgolides B.

Antioxidant properties

Ginkgo biloba extract (GBE) ameliorated the down-regulation of SOD and produced a significant reduction of MDA levels in spinal cord ischemia/reperfusion rabbits(Fan, Wang, and Cheng, 2006). MP and Ginkgo biloba treatments significantly decreased MDA levels. Furthermore, MP and Ginkgo biloba has a protective effect against ischaemic spinal cord injury via their

antioxidant effects in rat model(Koc et al., 1995). The research demonstrated that iNOS expression was upregulated after SCI. EGb761 can suppress iNOS expression and prevent neuronal death in SCI rats(Ao et al., 2006).

Anti-apoptotic properties

GBE was able to reduce the number of apoptotic cells, demonstrating its ability to reduce apoptosis. Levels of bcl-2 were up-regulated by GBE. The up-regulation of Bax was greatly diminished by GBE in rabbits). Analysis of apoptosis results and caspase-3 staining demonstrats that cell apoptosis was increased at 1–14 days post injury (DPI). At 7 DPI, the quantity of apoptotic cells significantly decreased in the EGb761-treated group in rats with acute spinal cord contusion injury(Yan et al., 2016). GBE inhibited nerve cell apoptosis via the mitochondrial caspase-dependent and caspase-independent pathways after spinal cord ischemia–reperfusion in rabbits. Furthermore, GBE could reduce levels of caspase-9 and apoptosis-inducing factor in the cytoplasm and serum(Cheng et al., 2011).

Neuroprotective properties

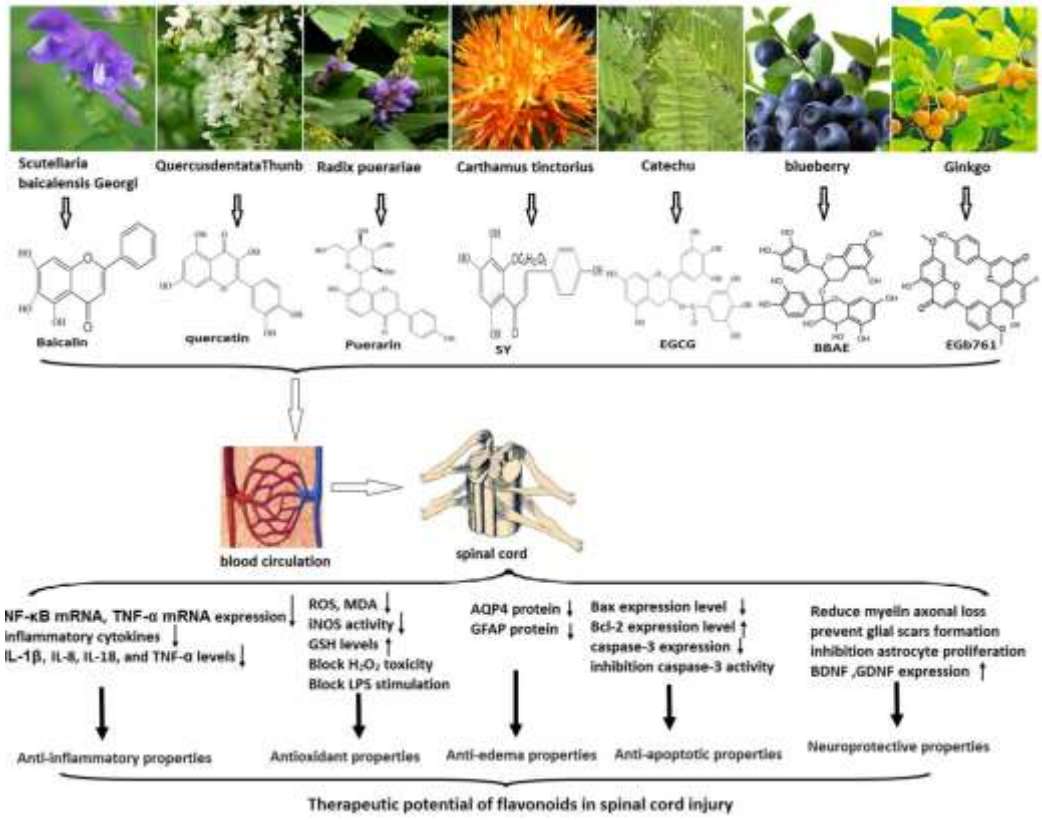
In a spinal cord ischemia/reperfusion study in rabbits, GBE improved the motor score and reduced lipid peroxidation and apoptosis(Fan, Wang, and Cheng, 2006). EGb761 protected spinal cord neurons against these insults indicating that such protection is mediated, at least in part, through the inhibition of cytosolic phospholipase A2 (cPLA2). The ERK1/2 signaling pathway mediated the activation of cPLA2, and treatment with EGb761 significantly decreased ERK1/2 phosphorylation. Thus, EGb761 may be considered as a potential therapeutic agent aiming at reducing secondary degeneration(Zhao et al., 2011).

Conclusion and future studies

In this paper, we reviewed therapeutic effects of different kinds of flavonoids (Fig 9), which mainly have anti-inflammatory, antioxidant, anti apoptosis and neuroprotective properties in treatment of SCI. The anti-inflammatory properties are mainly due to inhibiting the production of inflammatory cytokines. Flavonoids can reduce expression of NF-κB mRNA and TNF-α mRNA and decrease of IL-1β, IL-8, IL-18, and TNF-α levels. The antioxidative property of flavanoids in SCI is through reducing ROS, MDA, and iNOS activity, increasing the GSH levels, and blocking H_2O_2 toxicity and LPS stimulation. The anti-edema effect of flavanoid treatment after acute SCI is via down-regulation of the expression of AQP4 protein and GFAP proteins. Anti-apoptotic properties act mainly through the reduction of the expression of Bax, increase of the expression of Bcl-2 levels, and inhibition of caspase-3 expression and caspase-3 activity. Neuroprotective properties of the flavanoids after SCI work by reducing myelin axonal loss, glial scars formation, and astrocyte proliferation after SCI. Moreover, flavanoids could increase neurotrophic factors BDNF and GDNF in the spinal cords and decrease deactivationof growth signaling pathways.

Spinal cord injury may lead to neurological complications and eventually to paraplegia or quadriplegia. However, there is no effective treatment available, the main reason may be the complex pathophysiological mechanisms of spinal cord injury, and the secondary damage induced by a series of cellular and molecular events. Fortunately, flavonoids show promising therapeutic potential in spinal cord injury. Nevertheless, the molecular mechanisms involved in

TCM treatments still remain unclear, which require further research to reveal these. We hope that the TCM treatment combined with other treatments can treat and repair spinal cord injury in the near future, bringing key benefits to patients.



Acknowledgment

All authors declare that they have no competing interests.

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